

Quantum Yields of Soluble Particulate Material in the Ocean

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LONG-TERM GOALS

Our goal is to develop a more detailed understanding of the role of fluorescence as a source of trans-spectral energy in the underwater light field and the biological and chemical causes for variations in the spectral source terms. We will (i) determine the spectral quantum yield of cDOM and photosynthetic pigments; (ii) assess the impact of steady state growth conditions on phytoplankton pigment *in vivo* quantum yields; (iii) run model sensitivity analyses to determine the importance of trans-spectral sources in defining the submarine light field; and (iv) assess the implications of trans-spectral sources for hyperspectral remote sensors.

The proposed work is significant for the ONR optics program because no systematic, quantitative evaluation of the trans-spectral source terms have been accomplished and the quantum yields of the different components, which are essential for modeling and prediction, are poorly specified. The work is also of importance for the biology/chemistry programs since, despite the ubiquitous use of fluorescence as a tool to study cDOM and phytoplankton, the fundamental understanding of the processes in terms of quantum fluxes is not well known. We anticipate that our work will enhance the ability to predict optical, chemical and biological processes in the ocean.

OBJECTIVES

Our goal is to study the quantum yields of fluorescence of various components including dissolved, detrital particulate and phytoplankton. We will assess the role of fluorescence of these various components to contribute trans-spectral sources within the water column and environmental conditions that govern the fluorescence of biological and chemical components. Combining laboratory work and radiative transfer modeling, we will define which sources must be accounted for to understand the intensity of irradiance in the ocean for sources other than the natural solar flux and Raman. In year 1 of this project we focused on optical characterization of our spectral fluorometer, and the role of photoprotective pigments in modifying the spectral quantum yield of chlorophyll a fluorescence excitation in cultures of *Phaeocystis pouchetii*.

APPROACH

The fluorescence within the ocean can be described by the simple phenomenological description:

$$E_{Fi}(\lambda_m) = \int_{\lambda_m} \int_{\lambda_x} a_i(\lambda_x) \Phi_i(\lambda_x, \lambda_m) E_o(\lambda_x) d\lambda_x d\lambda_m \quad (1)$$

Where $E_{Fi}(\lambda_m)$ is the total trans-spectral fluorescence irradiance flux at wavelength λ_m for component i , $a_i(\lambda_x)$ is the absorption coefficient of component i at excitation wavelength λ_x , $\Phi_i(\lambda_x, \lambda_m)$ is the quantum yield for fluorescence of component i for the excitation and emission wavelengths, and

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$E_0(\lambda_x)$ is the scalar irradiance at the excitation wavelength. It is essential, then, to know the irradiance within the ocean, the absorption coefficients and the quantum yields of various components to fully describe the irradiance derived from fluorescence. Methods exist for determining the absorption coefficients and to measure the irradiance available to excite fluorescence. We need a better understanding of quantum yields to apply Equation 1, in a predictive way.

The quantum yield for fluorescence is defined as the ratio of the absorbed irradiance to the fluoresced irradiance (by rearranging Equation 1). Absolute characterization of the excitation irradiance, the volume defined by the illumination and measuring beams, and the absolute quantum response of the detector optics typically are not known. Because of this fact, a common approach for determining the quantum yield of an unknown in analytical chemistry is to compare it to a compound with known quantum yields (Renschler and Harrah, 1983). This has significant advantages since it does not require an absolute optical characterization of the spectrofluorometer, and one can be somewhat careless with respect to optical geometry, and still get reasonable results. Still, it is of value to fully characterize the fluorometer radiometrically so that it is possible to determine directly the quantum yields to validate the comparison approach and to allow more versatility when the assumptions for its application are not met.

Instrument Characterization

We fabricated a series of field stops to create a collimated beam that enters the sample with a well defined geometry, isolated the sample from scattered or stray light and created a light trap consisting of a tube with baffles to capture the light that passes through the sample to further minimize stray light. We will iterate on this approach several times until we are satisfied that the geometry has been well specified. The spectral quantum response of the detector optics has been characterized using a standard lamp calibrator available from Spex, Instruments. Broad band quantum counters are available which effectively absorb all energy from the source, and emit with 100% quantum efficiency. The manufacturer recommends using Rhodamine-B which is effective over the range from 220-600 nm. However, since we desire spectral excitation ranging through the visible (to at least 700 nm) for the purpose of chlorophyll fluorescence excitation studies, we have used the quantum counter 2,7-bis-(diethyl-amino) phenazooxonium perchlorate ("Basic Blue" or Oxazine 1 perchlorate, laser grade available from Eastman Kodak; Kopf and Heinze, 1984; Sosik and Mitchell, 1995). Given the well-defined geometry based on custom-built field stops, and the spectrally calibrated detector optics using the 1908 Standard Lamp Assembly, the excitation source has been spectrally calibrated using the quantum counter dyes.

Experiments on cultures

In parallel with the work to characterize and develop an absolute calibration of our spectrofluorometer, we have carried out experiments on the spectral *in vivo* quantum yield of chlorophyll-a fluorescence excitation using cultures of *Phaeocystis* under light limitation. The culture work has been carried out by Scripps graduate student Tiffany Moisan.

Cultures of colonial *Phaeocystis antarctica* Karsten (CCMP 1374) were grown semi-continuously for 5-8 generations in f/2 medium (Guillard and Ryther 1962) at 4°C. Cultures were diluted with fresh medium as necessary to ensure that they were nutrient-replete and "optically-thin" to minimize light attenuation due to phytoplankton. Cultures were bubbled with cold (4°C) sterile air and

grown under continuous blue light at 7 intensities ranging from 14 to 542 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$. The culture system is described in Moisan and Mitchell (1999a).

Fluorescence excitation spectra, $F(\lambda)$, were obtained at ambient growth temperatures using 1 nm increments with the emission monochromator set at 730 nm (Neori et al. 1988, Sosik and Mitchell 1995). The quantum corrected spectra were scaled at the red peak (676 nm) of the fluorescence excitation spectra, $F(\lambda)$, to the corresponding peak of the whole cell *in vivo* chlorophyll-specific absorption spectrum,

$$a_{ps}^*(\lambda, \text{m}^2 (\text{mg chl } a)^{-1}) = \frac{F(\lambda)a_{ph}^*(676\text{nm})}{F(676\text{nm})} \quad (2)$$

Absolute fluorescence is defined as,

$$F_0 = G(\lambda)E_0(\lambda)a_{ph}(\lambda)\phi_f(\lambda) \quad (3)$$

where $G(\lambda)$ is the geometric correction for the instrument, $E_0(\lambda)$ is the spectral irradiance of the excitation source, $a_{ph}(\lambda)$ is the absorption by phytoplankton, and $\phi_f(\lambda)$ is the quantum yield for fluorescence. The geometry and the spectral irradiance of the excitation source is constant for each treatment but this work was completed before our instrument modifications and characterization, so these data are relative spectral quantum yields. We divide $a_{ps}(\lambda)$ by $a_{ph}(\lambda)$ to estimate the relative fluorescence yield by the following equation,

$$\phi_f = \frac{a_{ps}(\lambda)}{a_{ph}(\lambda)}. \quad (4)$$

High Performance Liquid Chromatography

Chlorophyll *a* concentration was estimated fluorometrically (Yentsch and Menzel 1963) and by high performance liquid chromatography (HPLC, Wright et al. 1991). The HPLC data includes photosynthetic and accessory pigments soluble in acetone extracts. Mycosporine-like amino acids (MAAs) were separated by reverse-phase isocratic HPLC on a Brownlee RP-8 column (Dunlap and Chalker 1986). MAAs were quantified using a secondary calibration which is based on a known set of standards.

WORK COMPLETED

We have built the light baffle and light trap systems and installed them in our spectrofluorometer. Preliminary work with dyes of known quantum yields have been carried out and are encouraging. Mapping out the emission beam as it is projected to the emission monochromator and detector has revealed non-uniform distribution. We are attempting to modify the illumination stops and to add additional field stops to ensure a more uniform flux of energy onto the emission monochromator. We will rent the Spex calibrator periodically to determine the absolute calibration of our emission monochromator and detector.

We have carried out work to evaluate the role of photoprotective pigments including mycosporine amino acids and the xanthophyll pigments in *Phaeocystis* on the spectral quantum yield of chlorophyll *a* fluorescence.

RESULTS

We found that the fraction of photosynthetically active absorption determined from fluorescence excitation by Equation 2, was much lower than total absorption between 300 and 350 nm (Figure 1A). Using Equation 4, we estimated ϕ_f for the different cultures (Figure 1B). The low quantum yields of fluorescence between 300 nm to 350 nm suggests that a small fraction of the absorbed energy is transferred to chlorophyll *a*. We conclude based on fluorescence excitation spectra and knowledge of photosynthetic pigmentation in the UV region that most of the absorbed energy in the UV region is not transferred to the chlorophyll *a* and therefore does not participate in the photosynthetic process or fluorescence. It is most likely that MAAs act as a passive sunscreen which is supported by the observation that MAAs are located in the cytoplasm (Garcia-Pichel and Castenholz 1993) and are not coupled to either of the photosystems. We also observed a strong minimum in ϕ_f around 490 nm, the absorption maximum for photoprotective carotenoids that comprise the energy dissipating xanthophyll cycle in *Phaeocystis* (Moisan et al. 1998).

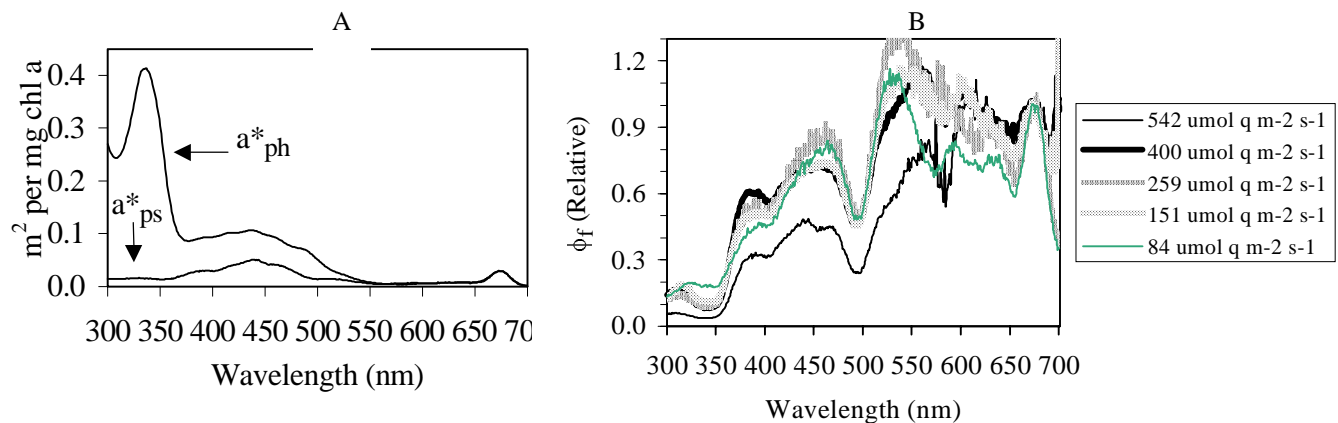


Figure 1. A). Example of chlorophyll *a* specific absorption for total phytoplankton (a^*_{ph}) and photosynthetically active pigments (a^*_{ps}). B). Estimates of $\phi_f(\lambda)$ for all cultures.

IMPACT/APPLICATIONS

The detailed UV-visible optical properties of photosynthetic pigments, including absorption and fluorescence, are fundamental to understanding variability of optical properties in the ocean. Knowledge of how light is absorbed, utilized and fluoresced by phytoplankton must be improved if we are to improve models of optical dynamics in the ocean including tran-spectral sources, which are governed in large part by the phytoplankton. The work describe here, and submitted for publication (Moisan et al. 1998, Moisan and Mitchell 1999b), demonstrates a strong spectral dependence in the quantum yield of fluorescence excitation of chlorophyll *a*. This type of information needs to be

incorporated into models of ocean optics so that the transfer function between absorbed energy, and the chlorophyll a trans-spectral source, can be more accurately characterized.

TRANSITIONS

Results of our work on *Phaeocystis* has been submitted for publication (Moisan and Mitchell, 1999a, Moisan and Mitchell, 1999b).

RELATED PROJECTS

Work in the last year has built on our previous ONR grants for optical studies (N00014-91J-1186) and microspectrophotometer instrumentation (N00014-94-1-0951). Many of the publications completed or submitted in the last year as referenced in the separate statistics attachment originated in our previous projects. They are cited here to demonstrate continuity of research accomplishments with the continuation of ONR funding. New work acknowledging these previous awards, not reported in last year's report and not cited above in this report, include 5 articles submitted to peer-reviewed journals and 2 articles submitted to Ocean Optics XIV.

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